PROJECT NUMBER: 6906
PROJECT TITLE: Biological Effects of Smoke
PROJECT LEADER: J. M. Penn
WRITTEN BY: L. H. Thompson and G. J. Patskan
PERIOD COVERED: September, 1988

I. INHIBITION OF EGF BINDING ASSAY

A. Objective: Determine the role of protein kinase C in CSC induced inhibition of EGF binding using protein kinase C inhibitors.

B. Results: To establish the optimal conditions for experiments with CSC, the effects of H-7 (a protein kinase C active site inhibitor) on the response to TPA and PDBu binding were determined. EGF binding was inhibited following a thirty minute exposure to TPA (100 nM) or PDBu (500 nM). H-7 (100 uM) had no effect on the response to TPA or PDBu.

C. Plans: Evaluate the effects of W-7, a protein kinase C inhibitor which acts at the the phorbol ester binding site, on the inhibition of EGF binding by TPA and PDBu.

D. Reference: Patskan, G. Notebook No. 8710, p. 1.

II. NICOTINE SPECIFIC MONOCLONAL ANTIBODY

A. Objective: To obtain a monoclonal antibody (MCA) against nicotine (NIC-MCA).

B. Results: The contract lab reported that the preparation and analysis of immune sera from mice injected with nicotine conjugated antigen has been completed. The results from an ELISA assay indicate that successful immunization has occurred. Those sera dilutions are being sent to PM for further analysis.

C. Plans: Examine the sera dilutions from the contract lab in an ELISA assay here at PM and determine the titers of all sera.

D. Reference: Davies, B. D. Notebook No. 8638, p. 70.

III. APPROACHES TOWARD PUTRESCINE METHYLTRANSFERASE (PMT) ISOLATION

A. Objective: Provide additional experimental approaches to assist in the effort to isolate PMT.

B. Results: A non-homogeneous preparation of phenethanolamine-N-methyl transferase (PET), an enzyme related to PMT, was subjected to SDS-PAGE (sodium dodecyl sulfate - polyacrylamide gel electrophoresis) and transferred to nitrocellulose. Immunostaining of the nitrocellulose with an antibody against PET (APET) revealed a specific band of ~29,000 MW. When PMT Prep I fractions were analyzed by the same procedure, nonspecific staining of all

proteins was observed with both APET and an antibody to a protein not found in plants. Also, densitometry of Coomassie stained gels of PMT Prep I fractions indicated an inverse correlation between the levels of protein in the PMT-containing region and the specific activity of the fraction. This indicated the possibility that this area still contains several contaminating proteins.

C. Plans: The nitrocellulose blotting procedure will be optimized to provide a more quantitative transfer of protein. The use of APET staining of PMT preparations will be discontinued until purer preparations of PMT become available. The number of proteins in the PMT containing region will be estimated using two dimensional gel electrophoresis.

D. Reference: Nixon, G.M. Notebook No. 8711, p. 1.

IV. GLUTATHIONE DEPLETION ASSAY (GDA)

A. Objective: To determine the effect of the reduction in GSH level by 2R1 CSC and diethyl maleate (DEM) on Salmonella TA98 activity of a direct acting control compound, 2NF.

B. Results: GSH in TA98 was depleted by both DEM and 2R1 CSC (27 and 51% respectively) and by each in the presence of 2NF (81% for 2R1 CSC + 2NF and 63% for DEM + 2NF). However, there was no increase in the TA98 2NF activity due to GSH depletion by either DEM or 2R1 CSC.

C. Plans: Plans are to repeat these experiments using [1-chloro-2,4-dinitrobenzene (CDNB)] as the direct acting compound.

D. Reference: McCoy, W. R. Notebook No. 8555, pp. 165-169.

V. SALMONELLA/MICROSOME (S/M ASSAY) -- CALCIUM EFFECTS ON SAMPLES FROM THE CROSSED SOLUBLE/BASE WEB STUDY

A. Objective: Various amounts of calcium acetate (to achieve a 3-7% range of total calcium) were added to either bright baseweb (BrBW) or burley CEL (BuCEL) oversprayed onto BrBW to determine: a) the effects on IT CSC delivery and/or burn rate and, b) if there was a concentration response towards activity in the S/M assay. An additional objective was to determine if the S/M IT CSC specific activity (S.A.) of the BuCEL + BrBW control could be altered by varying the amount of BuCEL added to the BW.

B. Results: Several conclusions were drawn from the data: 1) as percent Ca++ increased, CSC yields correspondingly decreased for both BrBW alone and BuCEL + BrBW; 2) there was no change in puff count when Ca++ was added to the BrBW; 3) when Ca++ was added to BuCEL and oversprayed onto the BW, the cigarettes with the highest amount of added Ca++ (7%) were difficult to smoke (20-22 puffs vs 10 puffs for the control); 4) Ca++ added to the BW alone did not produce a significant increase in S/M IT CSC S.A. as compared to

the control except for the higher CA++ (6-7%) levels; 5) BuCEL + BrBW with added Ca++ produced CSCs with higher S.A.s than their respective controls; however, the differences were not significant in all cases; 6) as anticipated, there were no distinct patterns to indicate a concentration response of added Ca++ in regards to S/M activity; and 7) as the amount of added BuCEL increased, S/M activity IT CSC S.A. generally increased.

C. Plans: An experiment is being performed which will determine the effect Ca++ has on the S/M activity of the CSC from BuCEL + BrBW filler that has a constant solids add-on weight. A Special Report will then be prepared to document all salt-effect studies.

D. Reference: Thompson, L. H. Notebook No. 8628, pp. 185-186.